Feb 03, 2023

# Stereologic Cell Population Estimates in Cleared Human Brain Tissue

DOI
dx.doi.org/10.17504/protocols.io.4r3l273dqg1y/v1

Bridget Wicinski[1], Patrick.hof[1]

[1]Icahn School of Medicine at Mount Sinai

B Bridget Wicinski



OPEN ACCESS

**DOI:** https://dx.doi.org/10.17504/protocols.io.4r3l273dqg1y/v1

**Protocol Citation:** Bridget Wicinski, Patrick.hof 2023. Stereologic Cell Population Estimates in Cleared Human Brain Tissue. **protocols.io** https://dx.doi.org/10.17504/protocols.io.4r3l273dqg1y/v1



**Protocol status:** Working
**We use this protocol and it's working**

**Created:** January 28, 2023

**Last Modified:** February 03, 2023

**Protocol Integer ID:** 75991

**Keywords:** stereologic cell population estimate, step protocol for stereologic assessment, stereologic assessment, stereologic analysis, stereologic tool, mbf bioscience stereo investigator cleared tissue software, region of cortex, analysis for distinct neuronal population, numerical quantities of various morphologic parameter, cleared human brain tissue, neuronal density, human brain tissue, contouring of cortical layer, neuronal densities in each layer, human cerebral cortex, cleared postmortem specimen, systematic sampling, various morphologic parameter, surface area, distinct neuronal population, volume of reference, neuronal population, laminar surface area, contouring, object throughout the analysis, sampling, cortical layer, cortex, sampling design, large specimen, object under study, encompassing broca, sampling parameter, slice from large specimen, brain, defined region, specific cytoplasmic marker, counting frame size

**Funders Acknowledgements:**
**Patrick Hof**
Grant ID: U01 MH117023-01

## Abstract

We provide a step-by-step protocol for stereologic assessment in the human cerebral cortex using cleared postmortem specimens with a laminar level of analysis for distinct neuronal populations identified by immunolabeling for specific cytoplasmic markers. Stereologic tools provide a set of simple rules and formulas to estimate numerical quantities of various morphologic parameters in tissue, with precision, accuracy and a design free of observer-induced bias. Parameters such a number, density, volume, surface area, or length can be easily estimated based on the systematic sampling of a region of interest (e.g., a layer of a cytoarchitecturally defined region of cortex, defined as a “volume of reference”), with an observer-independent random design, that gives each object under study (e.g., a neuronal population identified by a specific protein marker) the same probability to be sampled once and only once in its volume of reference, based on strict sampling criteria that are kept constant for a given object throughout the analysis. Stereologic analysis was performed on cleared slice from large specimens encompassing Broca’s area (Brodmann’s area 44/45), up to 4×4×2 cm, imaged at 3×3×3 µm pixel dimensions, and immunostained for NeuN, calretinin, and somatostatin, using the MBF Bioscience Stereo Investigator Cleared Tissue software (version 2022.1.1) with an Optical Fractionator design. Data were recorded as estimates of total number of neurons as well as neuronal densities in each layer. The protocol provides the detail of the sampling parameters including counting frame size, grid size, and disector height, definition and contouring of cortical layers and use of their boundaries to estimate laminar surface areas and volume. Full details on software operation and sampling design are explained.

## Attachments

PDF

Protocol_SI-CTE.pdf
797KB

## Troubleshooting